Biodiversity of *Dickeya* spp. Isolated from Potato Plants and Water Sources in Temperate Climate

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Abstract

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Bacteria from the genera *Dickeya* (formerly *Erwinia chrysanthemi*) and *Pectobacterium* (formerly *E. carotovora*) are the agents of blackleg and soft rot on many important crops. In 2005, *Dickeya solani* was isolated for the first time in Poland from a symptomatic potato plant. To establish the presence and diversity of *Dickeya* spp. in Poland, we surveyed potato fields and water sources, including surface waters near potato fields and water from potato-processing facilities and sewage plants. Only

D. dianthicola and *D. solani* were isolated from symptomatic potato, and only *D. zeae* and *D. chrysanthemi* were isolated from water sources. The *Dickeya* spp. isolated from potato formed a relatively homogenous group, while those from water sources were more diverse. To our knowledge, this is the first comprehensive characterization of *Dickeya* spp. isolated during several years from regions with a temperate climate in Central Europe.

Bacteria belonging to the genera *Dickeya* (formerly *Erwinia chrysanthemi*) and *Pectobacterium* (formerly *E. carotovora*) are pathogens of many economically important crops. These species have been included among the 10 most important bacterial plant pathogens based on their economic or scientific impact (Mansfield et al. 2012). The genus *Dickeya* has gone through major classification changes since Samson et al. (2005) assigned *E. chrysanthemi* to a novel genus, *Dickeya*, and divided it into six species: *Dickeya chrysanthemi*, *D. dadantii*, *D. dianthicola*, *D. dieffenbachiae*, *D. paradisiaca*, and *D. zeae*. More recently, *D. dieffenbachiae* was renamed *D. dadantii* subsp. *dieffenbachiae* (Brady et al. 2012) and two new species were described: *D. aquatica* and *D. solani* (Parkinson et al. 2014; van der Wolf et al. 2014). Currently, the genus *Dickeya* comprises seven genomospecies, but several reports suggest that new species of *Dickeya* may be described in the future (Palacio-Bielsa et al. 2010; Suharjo et al. 2014).

The pathogenic species of *Dickeya* and *Pectobacterium* are responsible for blackleg and soft rot. Blackleg symptoms include stem rot that can spread from the rotting seed tubers. It leads to leaf wilt, decreased growth, collapse of the plant, or even a complete lack of plant emergence (Pérombelon 2002). Soft rot symptoms include the odorless maceration of plant tissue, and the disease can develop both in the field and during storage.

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GenBank accession numbers for new gene sequences reported here are KR135078 to KR135112. The *D. solani* strains IFB0099 (LMG28824), IFB0158 (LMG28826), and IFB0168 (LMG28827); *D. dianthicola* strains IFB0157 (LMG28825) and IFB0485 (LMG28834); *D. zeae* strains IFB0189 (LMG28828), IFB0280 (LMG28829), IFB0329 (LMG28832), and IFB0334 (LMG28833); and *D. chrysanthemi* strains IFB0289 (LMG28830) and IFB0320 (LMG28831) were deposited in BCCM/LMG Belgian Collection of Microorganisms.

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Dickeya spp. are pathogens of both monocotyledons and dicotyledons (Ma et al. 2007). *Dickeya* spp. cause symptoms only under favorable conditions but can survive in plant tissue for long periods (Pérombelon 2002). In Europe, *Dickeya* spp. are pathogens of a wide range of hosts, including potato, ornamental plants, and herbs (Toth et al. 2011). Several countries have undertaken preventive measures to reduce the spread of *D. solani*. For instance, Scotland introduced a “zero-tolerance” policy for *Dickeya* spp. in potato production systems in 2010 (Elphinstone and Toth 2007), while Jamaica has listed *Dickeya* spp. as a quarantine pest (<http://www.moa.gov.jm/PlantHealth/index.php>) and prohibited import of seed potato from The Netherlands and Belgium. Moreover, *Dickeya* spp. have become a quarantine pest in Israel (Tsrer (Lahkim) et al. 2012). In Asia, the most prevalent species of *Dickeya* seems to be *D. zeae*, which has been reported to cause rice foot rot, banana soft rot, and bacterial stalk rot of maize (Myung et al. 2010; Pu et al. 2012; Zhang et al. 2014). *D. solani* causes economic loss in Israel. In this country, *Dickeya* spp. can spread and multiply rapidly because temperatures are high and fields are irrigated (Tsrer (Lahkim) et al. 2009).

D. solani has been detected in potato plants in several European countries (Sarris et al. 2011; Ślawiak et al. 2009a; Toth et al. 2011, van der Wolf et al. 2014) but the existence of *Dickeya* spp. (and especially *D. solani*) in waterways and other water sources has not been extensively studied. Most species found in water samples were identified as *D. zeae* but a few were identified as *D. aquatica*, *D. chrysanthemi*, *D. dianthicola*, *D. solani*, or an unidentified clade of DUC-2 (MK-7), (Laurila et al. 2008, 2010; Palacio-Bielsa et al. 2010; Parkinson et al. 2009, 2014; Pritchard et al. 2013b). Researchers have yet to determine whether *Dickeya* spp. can spread through waterways from country to country. To date, *D. aquatica* strains have been isolated only from the waterways of Scotland and Finland (Parkinson et al. 2014).

In 2005, *D. solani* was isolated for the first time in Poland; the bacterium was isolated from a symptomatic potato plant originating from a potato seed plantation (Ślawiak et al. 2009a). This detection of *D. solani* and the general lack of information concerning *Dickeya* spp. in Poland motivated us to conduct a detailed ecological survey for *Dickeya* spp. in potato plantations and waterways and other water sources in Poland.

Here, we report the results of a 4-year survey of potato plantations and water sources for *Dickeya* spp. The *Dickeya* spp. isolated from both plant and water samples were subjected to molecular

Table 1. Reference *Dickeya* strains used in this study, including type strains and strains previously isolated from water in various European countries other than Poland

Number	Species	IFB collection number	Other collection numbers	Country, year of isolation	Origin	Tuber maceration assay ^a	PFGE profile ^b	Reference ^c
1	<i>Dickeya aquatica</i>	IFB0153	Dw0431	Finland	Water	...	29	Laurila et al. 2008
2	<i>D. aquatica</i>	IFB0154	Dw0440	Finland, 2005	Water	...	30	Laurila et al. 2008, Parkinson et al. 2014
3	<i>D. aquatica</i>	IFB0155	Dw054	Finland, 2005	Water	...	31	Laurila et al. 2008, Parkinson et al. 2014
4	<i>D. aquatica</i>	IFB0156	Dw0512	Finland, 2005	Water	...	32	Laurila et al. 2008
5	<i>D. chrysanthemi</i> pv. <i>chrysanthemi</i> ^T	IFB0055	NCPBP402 ^T , CFBP2048 ^T , IPO2118	United States, 1958	<i>Chrysanthemum morifolium</i>	0.14 (± 0.09)	20	Samson et al. 2005
6	<i>D. dadantii</i>	IFB0003	-	-	-	...	51	IFB
7	<i>D. dadantii</i> ^T	IFB0010	NCPBP898 ^T , CFBP1269 ^T , IPO2120, SCRI1269	Comoros, 1960	<i>Pelargonium capitatum</i>	1.24 (± 0.23)	17	Samson et al. 2005
8	<i>D. dadantii</i>	IFB0016	3937	France	<i>Saintpaulia</i>	...	18	Kotoujansky et al. 1982
9	<i>D. dadantii</i> subsp. <i>dieffenbachiae</i> ^T	IFB0113	NCPBP2976 ^T , CFBP2051 ^T , IPO2125	United States, 1957	<i>Dieffenbachia</i> spp.	1 (± 0.23)	24	Samson et al. 2005
10	<i>D. dianthicola</i> ^T	IFB0103	NCPBP453 ^T , CFBP1200 ^T , IPO2114, SCRI4073	United Kingdom, 1956	<i>Dianthus caryophyllus</i>	1.6 (± 0.053)	2	Samson et al. 2005
11	<i>D. dianthicola</i>	IFB0188	IPO1747	Netherlands, 1992	<i>Solanum tuberosum</i>	...	2	PRI, J. M. van der Wolf
12	<i>D. paradisiaca</i> ^T	IFB0117	NCPBP2511 ^T , CFBP4178 ^T , IPO2129	Colombia, 1970	<i>Musa paradisiaca</i>	1.19 (± 0.38)	15	Samson et al. 2005
13	<i>D. solani</i> ^T	IFB0123	IPO2222, NCPBP4479 ^T	Netherlands, 2007	<i>S. tuberosum</i>	3.5 (± 0.54)	1	van der Wolf et al. 2014
14	<i>D. solani</i>	IFB0148	River 2-1	Scotland	Water	...	1	SASA, G. Saddler
15	<i>D. solani</i>	IFB0272	MK16, DUC-1	Scotland	Water	...	1	Pritchard et al. 2013a,c
16	<i>D. zea</i>	IFB0031	IPO946	Netherlands	<i>S. tuberosum</i>	...	19	PRI, J. M. van der Wolf
17	<i>D. zea</i>	IFB0067	IPO824	Israel	Water	...	21	PRI, J. M. van der Wolf
18	<i>D. zea</i>	IFB0087	IPO647	Australia	<i>S. tuberosum</i>	...	22	Samson et al. 2005
19	<i>D. zea</i>	IFB0110	IPO2121, NCPBP1121, CFBP1278	Malaysia 1961	<i>Ananas comosus</i>	1.13 (± 0.35)	23	Samson et al. 2005
20	<i>D. zea</i> ^T , phylotype II	IFB0119	IPO2131, NCPBP2538 ^T , CFBP2052 ^T	United States, 1970	<i>Zea mays</i>	0.59 (± 0.2)	25	Samson et al. 2005
21	<i>D. zea</i>	IFB0120	IPO2132, NCPBP2339, CFBP4176	United States, 1970	<i>C. morifolium</i>	...	15	Samson et al. 2005
22	<i>D. zea</i>	IFB0121	IPO2133, CFBP6466	Martinique, 1991	<i>A. comosus</i>	...	26	Samson et al. 2005
23	<i>D. zea</i>	IFB0138	IPO3329, NCPBP3531	Australia	<i>S. tuberosum</i>	...	16	van der Wolf et al. 2014
24	<i>D. zea</i>	IFB0216	IPO650	Australia, 1980	<i>S. tuberosum</i>	...	33	Parkinson et al. 2009
25	<i>D. zea</i>	IFB0217	IPO651	Australia, 1980	<i>S. tuberosum</i>	...	16	Parkinson et al. 2009
26	<i>D. zea</i>	IFB0218	RW117	England	Water	...	34	FERA, J. Elphinstone
27	<i>D. zea</i>	IFB0219	RW157	England	Water	...	35	FERA, J. Elphinstone
28	<i>D. zea</i>	IFB0220	RW192, P7246, IPO3495	England	Water	...	35	FERA, J. Elphinstone Pritchard et al. 2013b
29	<i>Dickeya</i> sp.	IFB0147	River1-1	Scotland	Water	...	27	SASA, G. Saddler
30	<i>Dickeya</i> sp.	IFB0149	River3-1	Scotland	Water	...	28	SASA, G. Saddler
31	<i>Dickeya</i> sp.	IFB0275	MK7, DUC-2	Scotland	Water	...	27	Pritchard et al. 2013b SASA, G. Saddler
32	<i>Dickeya</i> sp.	IFB0277	CITA A-3	Spain, 2005	Water	...	36	Palacio-Bielsa et al. 2010

^a Values are the means ± standard error ($n = 6$) of the fresh mass (grams) of macerated tissue per tuber in the potato maceration assay; ... indicates that the assay was not performed for this isolate.

^b Numbers indicate pulse-field gel electrophoresis (PFGE) profiles obtained for the reference strains and isolates from water and potato plants isolated in Poland and other European countries.

^c IFB = the collection of Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Gdansk, Poland; PRI = the collection of Plant Research International, Wageningen, The Netherlands; FERA = the collection of Fera Science Ltd., York, United Kingdom; and SASA = the collection of Science and Advice for Scottish Agriculture, Edinburgh, United Kingdom.

characterization. Moreover, the maceration ability of *Dickeya* isolates was determined on potato tubers. To our knowledge, this is the first long-term study of *Dickeya* spp. in potato fields and water sources in the temperate region of Central Europe.

Materials and Methods

Bacterial strains, media, and culture conditions. Bacterial strains obtained from international collections are listed in Table 1, while strains isolated and characterized during the current research are listed in Table 2. All strains were grown at 28°C on crystal violet pectate (CVP) medium (Hyman et al. 2001), Luria broth agar (LA), or in Luria broth Sambrook et al. 2000) for 24 to 48 h, unless otherwise stated. In liquid cultures, bacteria were grown with agitation (200 rpm; INFORS AGCH-4103).

Collection of symptomatic potato samples. *Dickeya* spp. in potato fields in Poland were assessed in the growing seasons of 2009, 2010, 2011, and 2013. During these years, we obtained 768 samples of either symptomatic potato stems ($n = 546$) or tubers ($n = 222$). In 27 cases, both potato stems and tubers were tested for pectinolytic *Dickeya* spp. The majority ($n = 691$) of symptomatic potato samples was obtained by Regional (Voivodeship) Inspectorates of the Main Inspectorate of Plant Health and Seed Inspection (MIPH&SI), Warsaw, Poland. Samples were collected during standard inspections of potato seed plantations by MIPH&SI inspectors each year between May and December; 24.5% of the samples were collected in June,

56.2% in July, and 7.7% in August. Potato tubers obtained later than September were from potato storage facilities. Another 77 samples were acquired from the following institutions: Plant Breeding and Acclimatization Institute–Potato Breeding Zamarte, Jadwisin and Młochów Research Center; Plant Breeding in Szyldak Sp. z o. o.; Pomeranian-Mazurian Potato Breeding in Strzeczcin Sp. z o. o.; and Wrocław University of Environmental and Life Sciences, Wrocław, Poland. The samples were collected from 98 cultivars but 43% of the samples were from the following seven cultivars: ‘Denar’ (8.7%), ‘Vineta’ (7.8%), ‘Irga’ (7.3%), ‘Satina’ (7.0%), ‘Lord’ (5.3%), ‘Innovator’ (3.4%), and ‘Bryza’ (3.3%). For 4.8% of the potato samples, no information was obtained about potato cultivar. After collection, each sample was placed in a plastic bag, tightly sealed, and sent (along with completed questionnaires) in a padded envelope to our laboratory. Most samples reached our laboratory within 2 days. The questionnaire included date and place of collection, potato cultivar, weather conditions, and the kind and amount of fertilizers used in the field.

Collection of water samples. In total, 7,902 water samples were obtained from the Central Laboratory (Torun, Poland) of MIPH&SI in 2010, 2011, 2012, and 2013. The samples were collected by the inspectors as part of the routine inspection for *Ralstonia solanacearum*, as described in EU Commission Directive 2006/63/CE. Samples were collected twice each year: in June to July and in August to September.

Table 2. *Dickeya* spp. isolated from potato and water samples in Poland

Isolate ^a	Species	IFB number ^b	Year of isolation	Potato cultivar ^c	Plant part ^c	Potato maceration assay ^d	PFGE profile ^e
Potato							
1	<i>D. dianthicola</i>	IFB0157	2009	Lord	Stem	0.94 (± 0.32)	2
2	<i>D. dianthicola</i>	IFB0485	2013	Igor	Stem	0.44 (± 0.13)	3
3	<i>D. dianthicola</i>	IFB0486	2013	Bellarosa	Tuber	1.21 (± 0.3)	3
4	<i>D. solani</i>	IFB0099	2005	Santa	Stem	3.13 (± 0.26)	1
5	<i>D. solani</i>	IFB0158	2009	Kuras	Stem	3.19 (± 0.71)	1
6	<i>D. solani</i>	IFB0167	2009	Fresco	Stem	2.09 (± 0.51)	1
7	<i>D. solani</i>	IFB0168	2009	Fresco	Stem	2.19 (± 0.14)	1
8	<i>D. solani</i>	IFB0169	2009	Innovator	Stem	2.94 (± 0.3)	1
9	<i>D. solani</i>	IFB0203	2010	Amora	Stem	2.32 (± 0.32)	1
10	<i>D. solani</i>	IFB0204	2010	Jelly	Stem	2.81 (± 0.56)	1
11	<i>D. solani</i>	IFB0214	2010	Kuras	Stem	3.85 (± 0.36)	1
12	<i>D. solani</i>	IFB0296	2011	...	Stem	2.69 (± 0.53)	1
13	<i>D. solani</i>	IFB0300	2011	Wiarus	Stem	2.93 (± 0.4)	1
14	<i>D. solani</i>	IFB0304	2011	Verne	Stem	3.01 (± 0.49)	1
15	<i>D. solani</i>	IFB0309	2011	Berber	Stem	3.13 (± 0.54)	1
16	<i>D. solani</i>	IFB0311	2011	Innovator	Stem	3.85 (± 0.7)	1
17	<i>D. solani</i>	IFB0313	2011	Innovator	Stem	3.08 (± 0.43)	1
18	<i>D. solani</i>	IFB0318	2011	Innovator	Stem	...	1
19	<i>D. solani</i>	IFB0458	2013	Bellarosa	Stem	3.27 (± 0.35)	1
20	<i>D. solani</i>	IFB0487	2013	Vineta	Stem	2.87 (± 0.42)	1
Water							
21	<i>D. chrysanthemi</i>	IFB0283	2011	Na	Na	...	39
22	<i>D. chrysanthemi</i>	IFB0284	2011	Na	Na	...	40
23	<i>D. chrysanthemi</i>	IFB0287	2011	Na	Na	...	4
24	<i>D. chrysanthemi</i>	IFB0288	2011	Na	Na	...	4
25	<i>D. chrysanthemi</i>	IFB0289	2011	Na	Na	...	6
26	<i>D. chrysanthemi</i>	IFB0320	2011	Na	Na	0.3 (± 0.09)	4
27	<i>D. chrysanthemi</i>	IFB0321	2011	Na	Na	...	4
28	<i>D. chrysanthemi</i>	IFB0336	2011	Na	Na	...	48
29	<i>D. zeae</i>	IFB0189	2010	Na	Na	1.56 (± 0.27)	7

(continued on next page)

^a *Dickeya* isolates from potato plants (Potato) or water.

^b IFB0099 (101A/9/2005, IPO2276) was isolated by Ślawiak et al. (2009a,b) and IFB0158 was isolated by Potrykus et al. (2014a). All others strains were isolated in the current study and some of them were deposited at BCCM/LMG: *D. chrysanthemi* IFB0289 (LMG28830), IFB0320 (LMG28831); *D. dianthicola* IFB0157 (LMG28825), IFB0485 (LMG28834); *D. solani* IFB0168 (LMG28827); *D. zeae* IFB0189 (LMG28828), IFB0280 (LMG28829), IFB0329 (LMG28832), IFB0334 (LMG28833).

^c Na indicates not applicable to water isolates.

^d Values are the means ± standard error ($n = 6$) of the fresh mass (grams) of macerated tissue per tuber in the potato maceration assay; ... indicates that the assay was not performed for this isolate.

^e Numbers indicate pulsed-field gel electrophoresis (PFGE) profiles obtained for the reference strains and isolates from water and potato plants isolated in Poland and other European countries.

In 2010, samples were collected from the same location twice each year. Beginning in 2011, samples collected in August to September (the second sampling in the year) were collected only from the four southern voivodeships. The samples originated from rivers, lakes, ponds, drainage ditches, and other types of surface water located near potato plantations. Water samples were also collected from potato-processing plants (samples were untreated) and from a maximum of 10 sewage plants (samples were untreated) in each region. The water samples were stored in the dark at room temperature for approximately 1 to 2 weeks before they were transferred to our laboratory.

Bacteria detection and isolation from potato plant and water samples. Because new information concerning bacterial detection and taxonomy became available during the 5 years of monitoring, the methods differed depending on the year. In 2009, we isolated pectinolytic bacteria from homogenized potato plant tissue on CVP medium, as described by Potrykus et al. (2014b), and approximately 1,000 pectinolytic isolates were tested with ADE1 and ADE2 PCR primers specific for *Dickeya* spp. (Nassar et al. 1996). Beginning in 2010, the procedure proposed by Potrykus et al. (2014b) was followed. Briefly, total genomic DNA was obtained from the potato samples. The DNA was subjected to multiplex polymerase chain reaction (PCR) targeting *Dickeya* spp., *Pectobacterium atrosepticum*, and *P. carotovorum* subsp. *carotovorum* + *P. wasabiae* in one reaction. If *Dickeya* spp. DNA was detected in the sample, we attempted bacterial isolation. Each year, samples of homogenized potato tissues and *Dickeya* isolates were frozen in 40% glycerol and stored at -80°C . To standardize procedures, the frozen homogenates from 2009 were reanalyzed by subjecting total DNA to the multiplex PCR.

For water samples, pectinolytic bacteria were isolated on CVP medium and subjected to PCR (Nassar et al. 1996) or multiplex PCR (Potrykus et al. 2014b). In all, 10 water samples (5 ml each) were combined and vigorously mixed, and the pooled sample was placed in a 50-ml Falcon test tube. Then, 0.2 ml of each pooled sample was spread on CVP and incubated at 28 and 37°C . After 48 to 72 h, the CVP plates were assessed for the presence of pit-forming bacteria (i.e., pectinolytic bacteria). If a pooled sample was positive for pectinolytic bacterial colonies, the individual water samples that were used to obtain the pooled sample were spread on CVP medium, and pectinolytic bacteria were isolated. The pectinolytic isolates that were identified as *Dickeya* spp. by PCR (Nassar et al. 1996) or multiplex PCR (Potrykus et al. 2014b) were purified on CVP and LA media, frozen in 40% glycerol, and stored at -80°C .

Characterization and phylogenetic analysis of *Dickeya* isolates. Pectinolytic *Dickeya* isolates obtained from symptomatic potato samples or from waterways were subjected to genome-wide analysis by DNA restriction enzyme digestion with *Xba*I (restriction fragment length polymorphism [RFLP]) and pulsed-field gel electrophoresis (PFGE) and were grouped according to their PFGE profiles (Lee et al. 2006; Degefu et al. 2013). Assignment of strains with similar PFGE patterns to individual *Dickeya* spp. was based on sequencing of the *dnaX* housekeeping gene (Ślawiak et al. 2009b) and, in some cases, on sequencing of *recA* (Parkinson et al. 2009; Waleron et al. 2002), *gyrA* (van der Wolf et al. 2014), and *rpoS* (Waleron et al. 2002). The amplicons were purified with a Clean-Up kit (A&A Biotechnology) and sequenced from both ends by Genomed. Phylogenetic analysis was based on *dnaX* gene sequences that were

Table 2. (continued from preceding page)

Isolate ^a	Species	IFB number ^b	Year of isolation	Potato cultivar ^c	Plant part ^c	Potato maceration assay ^d	PFGE profile ^e
30	<i>D. zeae</i>	IFB0190	2010	Na	Na	...	7
31	<i>D. zeae</i>	IFB0191	2010	Na	Na	...	37
32	<i>D. zeae</i>	IFB0192	2010	Na	Na	2.84 ($\pm$ 0.47)	7
33	<i>D. zeae</i>	IFB0193	2010	Na	Na	...	7
34	<i>D. zeae</i>	IFB0194	2010	Na	Na	...	38
35	<i>D. zeae</i>	IFB0197	2010	Na	Na	...	8
36	<i>D. zeae</i>	IFB0280	2011	Na	Na	3.41 ($\pm$ 0.41)	9
37	<i>D. zeae</i>	IFB0281	2011	Na	Na	...	9
38	<i>D. zeae</i>	IFB0282	2011	Na	Na	...	9
39	<i>D. zeae</i>	IFB0285	2011	Na	Na	...	12
40	<i>D. zeae</i>	IFB0286	2011	Na	Na	...	12
41	<i>D. zeae</i>	IFB0290	2011	Na	Na	...	6
42	<i>D. zeae</i>	IFB0291	2011	Na	Na	...	6
43	<i>D. zeae</i>	IFB0292	2011	Na	Na	...	5
44	<i>D. zeae</i>	IFB0293	2011	Na	Na	0.63 ($\pm$ 0.33)	41
45	<i>D. zeae</i>	IFB0294	2011	Na	Na	...	5
46	<i>D. zeae</i>	IFB0295	2011	Na	Na	...	5
47	<i>D. zeae</i>	IFB0319	2011	Na	Na	...	42
48	<i>D. zeae</i>	IFB0322	2011	Na	Na	...	10
49	<i>D. zeae</i>	IFB0323	2011	Na	Na	...	10
50	<i>D. zeae</i>	IFB0324	2011	Na	Na	0.88 ($\pm$ 0.37)	13
51	<i>D. zeae</i>	IFB0325	2011	Na	Na	...	13
52	<i>D. zeae</i>	IFB0326	2011	Na	Na	...	8
53	<i>D. zeae</i>	IFB0327	2011	Na	Na	...	11
54	<i>D. zeae</i>	IFB0328	2011	Na	Na	...	11
55	<i>D. zeae</i>	IFB0329	2011	Na	Na	0.23 ($\pm$ 0.23)	14
56	<i>D. zeae</i>	IFB0330	2011	Na	Na	...	14
57	<i>D. zeae</i>	IFB0331	2011	Na	Na	...	43
58	<i>D. zeae</i>	IFB0332	2011	Na	Na	...	44
59	<i>D. zeae</i>	IFB0333	2011	Na	Na	...	45
60	<i>D. zeae</i>	IFB0334	2011	Na	Na	3.83 ($\pm$ 0.48)	46
61	<i>D. zeae</i>	IFB0335	2011	Na	Na	...	47
62	<i>D. zeae</i>	IFB0338	2011	Na	Na	...	5
63	<i>D. zeae</i>	IFB0339	2011	Na	Na	...	5
64	<i>D. zeae</i>	IFB0488	2013	Na	Na	1.21 ($\pm$ 0.4)	49
65	<i>D. zeae</i>	IFB0489	2013	Na	Na	1.72 ($\pm$ 0.5)	16
66	<i>D. zeae</i>	IFB0490	2013	Na	Na	2.95 ($\pm$ 0.31)	50

manually edited with Bioedit 7.2.5 (Hall 1999). The *dnaX* sequences of *P. atrosepticum* IPO980, *P. wasabiae* SCRI3193, and *P. carotovorum* subsp. *carotovorum* 3733 were used as outgroups. The tree was generated with the maximum-likelihood method using Phylogeny.lirmm.fr (Dereeper et al. 2008) and was edited with TreeGraph 2 (Stöwer and Müller 2010). *dnaX* sequences of *Dickeya* spp. reference strains were obtained from GenBank. The tree was submitted to TreeBase Database (www.treebase.org) with the reference number 17463.

Potato tuber maceration assay. ‘Lord’ potato tubers were inoculated with pectinolytic *Dickeya* isolates obtained from potato fields and water, as previously described (Hugouvieux-Cotte-Pattat et al. 2004). In brief, the potato tubers were washed in tap water and dried. Six to nine potato tubers, each containing one hole made with a sterile pipette tip, were inoculated with 5 µl of every bacterial strain suspension (5×10^6 CFU). Inoculated holes were then covered with mineral oil to provide anaerobic conditions. After 48 h at 28°C, the rotted potato tissue was removed and weighed without drying. The experiment was performed twice.

The weather conditions. Daily rainfall and temperature records during the years of monitoring in Poland were obtained from the Institute of Meteorology and Water Management, National Research Institute, Warsaw, Poland, as a result of standard procedure executed by the national hydrological and meteorological service. The daily rainfall and temperature records were used for monthly average sum of rainfall and monthly average temperature calculations for the territory of Poland and are presented in Supplementary Figure S1.

Data analysis. The ability of *Dickeya* isolates to macerate potato tissue was compared by analysis of variance followed by Tukey’s posthoc test. Statistica 10.0 StatSoft Polska was used for all statistical analyses. Data are presented as means $\pm$ standard error, and significance was determined at $P < 0.05$.

Results

Detection of *Dickeya* spp. Pectinolytic bacteria were detected in 65% of the potato samples. The percentage of potato samples in which pectinolytic bacteria were identified by multiplex PCR was 63% in 2009, 71% in 2010, 82% in 2011, and 42% in 2013. The percentage of potato samples in which *Dickeya* spp. were detected by multiplex PCR was 4.24% in 2009, 1.39% in 2010, 12.11% in 2011, and 2.54% in 2013. Over the 4 years of sampling, *Dickeya* spp. were detected in 39 (35 stem and 4 tuber samples) of 768 samples by multiplex PCR. *Dickeya* spp. alone were detected in 22 samples and *Dickeya* spp. along with *Pectobacterium* spp. were detected in 17 samples. Of the 39 potato samples positive for *Dickeya* spp. by the multiplex PCR assay, viable cells were isolated from 18 stem samples and 1 tuber sample (Table 2). From 1 to 10 *Dickeya* isolates from each potato sample were obtained. Voivodeships in which *Dickeya* spp. were identified on potato plants differed from year to year (Fig. 1). Over the 4 years of sampling, *Dickeya* spp. were detected in 11 of the 14 voivodeships that were surveyed.

Viable cells of pectinolytic *Dickeya* spp. were isolated from 28 of the 7,902 water samples (Table 2). The pectinolytic bacteria were isolated from 6 water samples in 2010, 19 in 2011, 0 in 2012, and 3 in 2013. In total, 46 *Dickeya* strains were isolated from 28 water samples. The geographical distribution of *Dickeya* spp. in water samples differed among years but *Dickeya* spp. were detected in most of the surveyed voivodeships (Fig. 1B).

Poland has a humid continental climate (a transient temperate climate), and weather conditions are likely to affect the development of blackleg or soft rot in the field. We examined the influence of weather conditions on the occurrence of *Dickeya* spp. in the potato fields. Using multiplex PCR, we detected *Dickeya* spp. in 12.11% of the samples in 2011 but in only 1.39% of samples in 2010. The average temperature in 2011 was the highest (8.7°C) in the investigated period whereas it was the lowest (7.3°C) in 2010. The average monthly rainfall was lower in 2011 (760 mm) than in 2010 (1,120 mm) but the amount of rainfall was 1.5-fold higher in July 2011 than in July 2010.

Overall, the highest number of *Dickeya* isolates were obtained in 2011 from both potato and water samples (Fig. 1). In total, 19

Dickeya isolates from potato plants and 46 from water samples were subjected to characterization.

***Dickeya* isolates from potato plants were *D. solani* or *D. dianthicola*.** We characterized *Dickeya* spp. isolated from 19 symptomatic potato plants (Table 2). RFLP-PFGE analysis was performed on all *Dickeya* isolates. In every case, profiles of strains derived from the same sample (the same plant) were identical (data not shown). For characterization of the *Dickeya* isolates, one representative isolate from each symptomatic plant was chosen. One of the first Polish strains of *D. solani* isolated in 2005 (IFB0099, 101A/9/2005) was used as a reference and, all together, 20 isolates from potato were assessed. The *Dickeya* isolates predominantly originated from symptomatic potato stems; only one isolate (IFB0486) was obtained from rotten potato tuber. PFGE profiling indicated that the isolates from potato samples clustered into three groups (Fig. 2; Table 2). Seventeen *D. solani* isolates (including the isolate from 2005) had PFGE profiles that were identical to that of the reference strain *D. solani* IPO2222 (NCPBB4479^T, IFB0123). One *Dickeya* isolate from 2009 (IFB0157) grouped together with the type strain of *D. dianthicola* (CFBP1200^T, IFB0103). In addition, two *Dickeya* isolates from 2013 (IFB0485 and IFB0486) had identical profiles that differed from the profiles of the *D. dianthicola* CFBP1200^T.

dnaX sequence data from 20 isolates were used to perform a phylogenetic analysis. The sequences of other genes were also used to identify isolates to species; *recA* was sequenced for five isolates, *rpoS* for three isolates, and *gyrA* for eight isolates. In the phylogenetic analysis based on *dnaX* sequences, all isolates previously identified by RFLP-PFGE analysis as *D. solani* grouped with the reference strains of this species (Fig. 3). *dnaX* sequences of *D. dianthicola* IFB0157, IFB0485, and IFB0486 were identical and grouped with the sequences of *D. dianthicola* CFBP1200^T even though the three isolates did not exhibit the same PFGE profile (Fig. 3). Altogether, we isolated 16 strains of *D. solani* that were identical both in RFLP-PFGE and in sequences of *dnaX*, *recA*, *rpoS*, and *gyrA*; and three strains that were classified as *D. dianthicola* based on *dnaX* sequence but that exhibited two different PFGE profiles. When all of the obtained sequences were compared with those in GenBank, all *D. solani* gene fragments were the same as in *D. solani* IPO2222, and the *D. dianthicola* fragments were the same as in *D. dianthicola* CFBP1200^T.

In the potato tuber assay, the ability of *D. solani* isolates from 2005, 2009, 2010, 2011, and 2013 to macerate plant tissue was similar to that of the *D. solani* reference strain IPO2222 (Fig. 4A; Tables 1 and 2). Maceration ability was higher for the tested *D. solani* isolates than for the tested *D. dianthicola* isolates and was intermediate for the reference strain *D. dianthicola* CFBP1200^T (Fig. 4A).

***Dickeya* isolates from water sources are more diverse than those from potato plants.** We obtained 46 pectinolytic *Dickeya* isolates from 28 water samples (Table 2). All 46 isolates were subjected to RFLP-PFGE analysis and *dnaX* gene sequencing. Moreover, *rpoS* was sequenced for four isolates and *recA* was sequenced for seven isolates. For comparison of diversity, PFGE analysis and housekeeping gene sequencing were also performed for *Dickeya* spp. isolated from water sources in other European countries (Fig. 5; Table 1). We included two isolates from Scotland for which draft genome sequences are available in GenBank (MK16 and MK7), three other isolates from Scotland (River1-1, River2-1, and River3-1), four isolates from Finland (Dw0431, Dw0440, Dw054, and Dw0512), three isolates from England (RW117, RW157, and P7246), and one isolate from Spain (CITA A-3) (Table 1). These European strains were previously identified as *D. aquatica*, *D. dadantii* subsp. *dieffenbachiae*, *D. solani*, and *D. zeae* (Palacio-Bielsa et al. 2010; Parkinson et al. 2014; Pritchard et al. 2013a,c) (Toth and Saddler, personal communication).

The 46 Polish *Dickeya* isolates from water exhibited 26 different PFGE profiles. Only a few of the PFGE profiles were identical. The profiles of the Polish isolates were also different from the profiles of *Dickeya* spp. reference strains (Figs. 2 and 5; Tables 1 and 2).

In the phylogenetic analysis based on *dnaX* sequences, the *Dickeya* isolates from water sources formed two clades. The first clade contained seven isolates from three water samples collected in

midwestern (IFB0283, IFB0284, IFB0287, IFB0288, and IFB0289) and southern (IFB0320 and IFB0321) Poland in 2011 (Fig. 3). Although this clade included *D. chrysanthemi* pv. *chrysanthemi* reference strain NCPPB402^T (IFB0055), PFGE profiles of these seven isolates differed from the one of reference strain NCPPB402^T. PFGE profiles were identical for isolates IFB0287, IFB0288, IFB0320, and IFB0321 but were unique for the other three isolates in this clade. The second clade of *Dickeya* isolates from water consisted of 39 isolates (Table 2) that grouped with *D. zeae* reference strains (Fig. 3). Again, the PFGE profiles were diverse, and only the profile of IFB0489 was identical to that of *D. zeae* IPO3329. Although *D. dianthicola* and

D. solani were isolated from water in other European countries (Laurila et al. 2008, Toth et al. 2011), they were not isolated from water in the current study in Poland. *D. aquatica* also was not detected in this survey.

We also examined PFGE profiles of European *Dickeya* isolates from water. Each Finnish *D. aquatica* isolate exhibited a unique but similar PFGE profile (Fig. 5) but these isolates were identical in terms of *dnaX* sequence (Fig. 3). The profile for River1-1 from Scotland was identical to that of MK7 from Scotland (Fig. 5), and they grouped into one clade based on *dnaX* phylogeny (Fig. 3). Among the three isolates from England, the PFGE profiles of

Fig. 1. Detection of *Dickeya* spp. in **A**, potato plants and **B**, water in Poland. Potato fields were sampled in 2009, 2010, 2011, and 2013. Water sources were sampled in 2010, 2011, 2012, and 2013. Regions from which inspectors did not provide any samples are indicated in gray. Listed colors indicate regions where the following *Dickeya* spp. were detected: orange = *Dickeya solani*, yellow = *D. dianthicola*, orange/yellow = both *D. solani* and *D. dianthicola*, blue = *D. zeae*, blue/black = both *D. zeae* and *D. chrysanthemi*. Green color was implemented for regions where no *Dickeya* spp. were detected.

Fig. 2. Restriction fragment length polymorphism pulsed-field gel electrophoresis (RFLP-PFGE) analysis of *Dickeya* spp. genomic profiles. Investigated strains were isolated from **A**, potato and **B**, water samples in Poland. Reference strains of *Dickeya* spp. were included. **A**, Polish strains isolated from potato formed three groups: the first group belongs to *Dickeya solani* while the other two groups belong to *D. dianthicola*. **B**, PFGE profiles indicate the substantial diversity of isolates from water samples. The size marker is the genomic DNA of *Salmonella enterica* ser. *braenderup* digested with *Xba*I.

RW157 and P7246 were identical but differed from that of RW117 (data not shown). Overall, the genetic diversity of *Dickeya* spp. from water sources was high among Polish and European isolates (Figs. 2 and 5).

The potato maceration assay was performed with 11 representative isolates from water that differed in PFGE profile and origin. For comparison, the assay included the following reference strains: *D. chrysanthemi* pv. *chrysanthemi* NCPPB402^T (IFB0055), *D. zeae*

Fig. 3. Phylogenetic analysis based on *dnaX* sequences of 20 *Dickeya* isolates from potato and 46 from water in Poland. The reference strains of *Dickeya* spp. and water isolates from other European countries are included in the analysis. The *dnaX* sequences of *Pectobacterium atrosepticum* IPO980, *P. wasabiae* SCRI3193, and *P. carotovorum* subsp. *carotovorum* 3733 were used as an outgroup. Bootstrap values are shown at the nodes. The tree is drawn to scale, with branch length reflecting evolutionary distance between strains. The tree was generated by the maximum-likelihood method with PhylogenyIimm.fr (Dereeper et al. 2008) and was edited with TreeGraph (Stöver and Müller 2010). IFB = the collection of Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Gdansk, Poland; IPO = the collection of Plant Research International, Wageningen, The Netherlands.

phylotype II CFBP2052^T (IFB0119), and CFBP1278 (IFB0110). The Polish *D. zeae* isolates from water were more virulent than the reference strains NCPPB402^T and CFBP2052^T (Fig. 4B). However, the maceration ability of Polish *D. chrysanthemi* strain IFB0320

was lower than that of *D. zeae* strains and was similar to that of *D. chrysanthemi* pv. *chrysanthemi* NCPPB402^T (Fig. 4B; Tables 1 and 2). Interestingly, the diversity evident in the PFGE profiles was also evident in the potato maceration assay (Tables 1 and 2).

Fig. 4. Virulence of *Dickeya* isolates from **A**, potato and **B**, water as indicated by a potato tuber maceration assay. Reference strains are indicated by gray bars. For statistical analysis, isolates were grouped by year and species for potato isolates ($F = 7.15$) and for water isolates ($F = 8.42$). Effect of group (species–year) was tested with an analysis of variance, and means were compared by Tukey's posthoc test. Values are means $\pm$ standard error ($n = 6$). In each panel, means with the same letter are not significantly different ($P > 0.05$). NCPBP402^T = *D. chrysanthemi* pv. *chrysanthemi*, CFBP1200 = *D. dianthicola*, IPO2222 = *D. solani*, CFBP1278 = *D. zeae*, and CFBP2052 = *D. zeae* phylotype II.

Fig. 5. Restriction fragment length polymorphism pulsed-field gel electrophoresis (RFLP-PFGE) profile analysis of water isolates from European countries (Scotland, Finland, Israel, and Spain) other than Poland. Reference strains of *Dickeya* spp. are included. The size marker used is the genomic DNA of *Salmonella enterica* ser. *braenderup* digested with *Xba*I.

The quantity of macerated tissue was 16 times greater for the most pathogenic *D. zea* strain (IFB0334) than for the least pathogenic *D. zea* strain (IFB0329) (Table 2).

Discussion

Bacteria belonging to the genus *Dickeya* cause blackleg and soft rot on many plants and are major problems in potato production (Czajkowski et al. 2011; Toth et al. 2011). We performed a 4-year survey of *Dickeya* spp. in potato seed plantations and waterways and other water sources in Poland. We detected *Dickeya* spp. in 39 (5%) of the 786 potato plant samples and in 28 (0.4%) of the 7,902 water samples.

Potato samples were analyzed as described by Potrykus et al. (2014b). Unfortunately, from 20 of the 39 potato plant samples in which *Dickeya* spp. DNA was detected, viable bacterial cells were not isolated. We suspect that the *Dickeya* spp. in the potato tissue did not survive transportation to our laboratory because of summer heat and rot caused by other bacteria or fungi. Another reason for the failure to isolate *Dickeya* spp. from PCR-positive samples could be coinfection with *Pectobacterium* spp. In 10 of 19 potato samples, extensive growth of *Pectobacterium* spp. on CVP plates interfered with the isolation of *Dickeya* spp.

Several studies of *Dickeya* spp. have been conducted in Europe over the last 10 years. In Spain, *Dickeya* spp. were detected in irrigation water and in potato and onion tissue (Palacio-Bielsa et al. 2010). In Finland, potato samples collected in 2004 and 2005 included 85 symptomatic stems and 23 rotten tubers (Laurila et al. 2008). Pectinolytic *Dickeya* spp. were isolated from 75% of symptomatic potato stems and 91% of symptomatic potato tubers. Strains belonging to *Dickeya* spp. were found in eight potato samples (Laurila et al. 2008). *Dickeya* spp. were also detected in seed potato tuber plantations in the High Grade zone in Finland (Degefu et al. 2013). In 2011 and 2012, *D. solani* was detected in symptomatic potato samples in The Netherlands, Denmark, Norway, Switzerland, Germany, and Czech Republic (EraNet Euphresco Report; J. van der Wolf and M. Bergsma Vlami, personal communication). Our findings indicate that *Dickeya* spp. are constantly present in Polish seed potato plantations, although the number of infected potato plants is low. We obtained 16 isolates of *D. solani* and 3 of *D. dianthicola* from potato samples. We did not isolate *D. solani* and *D. dianthicola* from the same potato plant, as reported in The Netherlands (R. Czajkowski, personal communication). However, we detected *Dickeya* spp. and *Pectobacterium* spp. in the same potato plant.

We also detected *Dickeya* spp. in water samples from Poland. Two *Dickeya* spp. were isolated from Polish water samples: *D. zea* and *D. chrysanthemi*. The presence of *Dickeya* spp. in water samples was also investigated in The Netherlands and England, where both *D. zea* and *D. solani* strains were isolated; however, *D. solani* was isolated sporadically, while *D. zea* was more abundant (EraNet Euphresco Report; J. van der Wolf and M. Bergsma Vlami, personal communication). *D. dianthicola* and *D. solani* have been isolated from water samples in other European countries (Laurila et al. 2008; Toth et al. 2011); however, these two species were not detected in Polish water samples in the current study. Moreover, we did not detect *D. aquatica*, which was recently detected in water samples in Finland and Scotland (Parkinson et al. 2014).

Overall, we obtained and characterized 19 *Dickeya* isolates from potato plants and 46 from water samples. PFGE profiling proved to be an efficient and reproducible method for determining isolate diversity. PFGE profiles for *Dickeya* isolates from the same potato plant were always identical. Also, all *D. solani* PFGE profiles were the same, suggesting homogeneity among this species regardless of isolate origin. In contrast to low biodiversity of the *Dickeya* strains isolated from potato (only three PFGE profiles: one for *D. solani* and two for *D. dianthicola*), high diversity was observed for *Dickeya* strains isolated from water (26 PFGE profiles: 4 for *D. chrysanthemi* and 22 for *D. zea*). Most water isolates were obtained from southern Poland, which is frequently flooded and is the main region for maize cultivation. According to phylogeny based on *dnaX*, most of our water isolates were *D. zea*. It would be useful to determine whether *D. zea* can be found in maize fields in Poland. The *dnaX*-based phylogeny of

the isolates enabled us to assign them to species, and such assignments were confirmed by analysis of *recA* sequences (data not shown).

Regarding the ability to macerate potato tissue under laboratory conditions, the *Dickeya* isolates from potato formed two groups: *D. solani* with high virulence and *D. dianthicola* with low virulence. Under our experimental conditions, the *D. dianthicola* isolates were even less virulent than *D. zea* water isolates. *D. zea* isolates were more virulent than the reference strain of this species, suggesting that *D. zea* represents a potential threat to potato production in Poland. However, the virulence of these isolates should be verified under greenhouse and field conditions.

Examination of the influence of weather conditions on the occurrence of *Dickeya* spp. in the potato fields indicated that the highest number of *D. solani* strains was detected in 2011, when the yearly average rainfall was the lowest and the yearly average temperature was the highest in comparison with 2009, 2010, and 2013. Our findings agree with Degefu et al. (2013), who suggested that the prevalence of *Dickeya* spp. is higher if the summer is hot and dry.

In summary, this is the first long-term, large-scale survey for *Dickeya* spp. in seed potato fields and water sources in Poland, which occupies a large portion of the temperate region of Central Europe. Only *D. solani* and *D. dianthicola* were isolated from symptomatic potato, and only *D. zea* and *D. chrysanthemi* were isolated from water sources. Genetic diversity of *Dickeya* strains was low for potato isolates but very high for water isolates. The diversity in PFGE profiles of *D. zea* strains matched their diversity in the virulence measured by the ability to macerate potato tissue. Polish *D. solani* strains indicated significantly higher potato maceration ability than *D. zea* strains, and very high genetic homogeneity.

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Literature Cited

- Brady, C. L., Cleenwerck, I., Denman, S., Venter, S. N., Rodríguez-Palenzuela, P., Coutinho, T. A., and De Vos, P. 2012. Proposal to reclassify *Brenneria quercina* (Hildebrand & Schroth 1967) Hauben et al. 1999 into a novel genus, *Lonsdalea* gen. nov., as *Lonsdalea quercina* comb. nov., descriptions of *Lonsdalea quercina* subsp. *quercina* comb. nov., *Lonsdalea quercina* subsp. *iberica* subsp. nov. and *Lonsdalea quercina* subsp. *britannica* subsp. nov., emendation of the description of the genus *Brenneria*, reclassification of *Dickeya dieffenbachiae* as *Dickeya dadantii* subsp. *dieffenbachiae* comb. nov., and emendation of the description of *Dickeya dadantii*. Int. J. Syst. Evol. Microbiol. 62:1592-1602.
- Czajkowski, R., Pérombelon, M. C. M., van Veen, J. A., and van der Wolf, J. M. 2011. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: A review. Plant Pathol. 60:999-1013.
- Degefu, Y., Potrykus, M., Golanowska, M., Virtanen, E., and Lojowska, E. 2013. A new clade of *Dickeya* spp. plays a major role in potato blackleg outbreaks in North Finland. Ann. Appl. Biol. 162:231-241.
- Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., and Gascuel, O. 2008. Phylogeny.fr: Robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36:W465-W469.
- Elphinstone, J., and Toth, I. K. 2007. *Erwinia chrysanthemi* (*Dickeya* species.) The Facts. British Potato Council Publications. Online publication. <http://potatoes.aahdb.org.uk/>.
- Hall, T. A. 1999. BioEdit: A user friendly biological sequence alignment editor and analysis program for Windows/p5/98/NT. Nucleic Acid Symp. Ser. 41:95-98.
- Hugouvieux-Cotte-Pattat, N. 2004. The *rhaS* activator controls the *Erwinia chrysanthemi* 3937 genes *rhlN*, *rhlT*, and *rhlE* involved in rhamnogalacturonan catabolism. Mol. Microbiol. 51:1361-1374.
- Hyman, L. J., Sullivan, L., Toth, I. K., and Pérombelon, M. C. M. 2001. Modified crystal violet pectate medium (CVP) based on a new polypectate source (Slendit) for the detection and isolation of soft rot erwinias. Potato Res. 44:265-270.
- Kotoujansky, A., Lemattre, M., and Boistard, P. 1982. Utilization of a thermosensitive episome bearing transposon TnJO to isolate Hfr donor strains of *Erwinia carotovora* subsp. *chrysanthemi*. J. Bacteriol. 150:122-131.
- Laurila, J., Ahola, V., Lehtinen, A., Joutsjoki, T., Hannukkalä, A., Rahkonen, A., and Pirhonen, M. 2008. Characterization of *Dickeya* strains isolated from potato and river water samples in Finland. Eur. J. Plant Pathol. 122:213-225.

- Laurila, J., Hannukkala, A., Nykyr, J., Pasanen, M., Hélias, V., Garland, L., and Pirhonen, M. 2010. Symptoms and yield reductions caused by *Dickeya* species strains isolated from potato and river water in Finland. *Eur. J. Plant Pathol.* 126:249-262.
- Lee, Y. A., Chen, K. P., and Hsu, Y. W. 2006. Characterization of *Erwinia chrysanthemi*, the soft-rot pathogen of white-flowered calla lily, based on pathogenicity and PCR-RFLP and PFGE analyses. *Plant Pathol.* 55:530-536.
- Ma, B., Hibbing, M. E., Kim, H. S., Reedy, R. M., Yedidia, I., Breuer, J., Breuer, J., Glasner, J. D., Perna, T., Kelman, A., and Charkowski, A. O. 2007. Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya*. *Phytopathology* 97:1150-1163.
- Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., and Foster, G. D. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.* 13:614-629.
- Myung, I. S., Jeong, I. H., Moon, S. Y., Kim, W. G., Lee, S. W., Lee, Y. H., Lee, Y. K., Shim, H. S., and Ra, D. S. 2010. First report of bacterial stalk rot of sweet corn caused by *Dickeya zeae* in Korea. *New Dis. Rep.* 22:15.
- Nassar, A., Darrasee, A., Lemattre, M., Kotoujansky, A., Dervin, C., Vedel, R., and Bertheau, Y. 1996. Characterization of *Erwinia chrysanthemi* by pectolytic isozyme polymorphism and restriction fragment length polymorphism analysis of PCR amplified fragment of *pel* genes. *Appl. Environ. Microbiol.* 62: 2228-2235.
- Palacio-Bielsa, A., Mosquera, M. E. R., Álvarez, M. A. C., Rodríguez, I. M. B., López-Solanilla, E., and Rodríguez-Palenzuela, P. 2010. Phenotypic diversity, host range and molecular phylogeny of *Dickeya* isolates from Spain. *Eur. J. Plant Pathol.* 127:311-324.
- Parkinson, N., DeVos, P., Pirhonen, M., and Elphinstone, J. 2014. *Dickeya aquatica* sp. nov., isolated from waterways. *Int. J. Syst. Evol. Microbiol.* 64: 2264-2266.
- Parkinson, N., Stead, D., Bew, J., Heeney, J., Tsrör (Lahkim), L., and Elphinstone, J. 2009. *Dickeya* species relatedness and clade structure determined by comparison of *recA* sequences. *Int. J. Syst. Evol. Microbiol.* 59:2388-2393.
- Pérombelon, M. C. M. 2002. Potato diseases caused by soft rot erwinias: An overview of pathogenesis. *Plant Pathol.* 51:1-12.
- Potrykus, M., Golanowska, M., Hugouvieux-Cotte-Pattat, N., and Lojkowska, E. 2014a. Regulators involved in *Dickeya solani* virulence, genetic conservation, and functional variability. *Mol. Plant-Microbe Interact.* 27:700-711.
- Potrykus, M., Sledz, W., Golanowska, M., Sławiak, M., Binek, A., Motyka, A., Zoledowska, S., Czajkowski, R., and Lojkowska, E. 2014b. Simultaneous detection of major blackleg and soft rot bacterial pathogens in potato by multiplex polymerase chain reaction. *Ann. Appl. Biol.* 165:474-487.
- Pritchard, L., Humphris, S., Baeyen, S., Maes, M., van Vaerenbergh, J., Elphinstone, J., Saddler, G. S., and Toth, I. K. 2013a. Draft genome sequences of four *Dickeya dianthicola* and four *Dickeya solani* strains. *Genome Announc.* 1:e00087-12.
- Pritchard, L., Humphris, S., Saddler, G. S., Elphinstone, J. G., Pirhonen, M., and Toth, I. K. 2013b. Draft genome sequences of 17 isolates of the plant pathogenic bacterium *Dickeya*. *Genome Announc.* 1:e00978-13.
- Pritchard, L., Humphris, S., Saddler, G. S., Parkinson, N. M., Bertrand, V., Elphinstone, J. G., and Toth, I. K. 2013c. Detection of phytopathogens of the genus *Dickeya* using a PCR primer prediction pipeline for draft bacterial genome sequences. *Plant Pathol.* 62:587-596.
- Pu, X. M., Zhou, J. N., Lin, B. R., and Shen, H. F. 2012. First report of bacterial foot rot of rice caused by a *Dickeya zeae* in China. *Plant Dis.* 96:1818.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 2000. *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Samson, R., Legendre, J. B., Christen, R., Fischer-Le Saux, M., Achouak, W., and Gardan, L. 2005. Transfer of *Pectobacterium chrysanthemi* (Burkholder et al. 1953) Brenner et al. 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. *Int. J. Syst. Evol. Microbiol.* 55:1415-1427.
- Sarris, P. F., Trantas, E., Pagoulidou, M., Stavrou, D., Ververidis, F., and Goumas, D. E. 2011. First report of potato blackleg caused by biovar 3 *Dickeya* sp. (*Pectobacterium chrysanthemi*) in Greece. *New Dis. Rep.* 24:21.
- Sławiak, M., Lojkowska, E., and van der Wolf, J. M. 2009a. First report of bacterial soft rot on potato caused by *Dickeya* sp. (syn. *Erwinia chrysanthemi*) in Poland. *Plant Pathol.* 58:794.
- Sławiak, M., van Beckhoven, J. R. C. M., Speksnijder, A. G. C. L., Czajkowski, R., Grabe, G., and van der Wolf, J. M. 2009b. Biochemical and genetical analysis reveal a new clade of biovar 3 *Dickeya* spp. strains isolated from potato in Europe. *Eur. J. Plant Pathol.* 125:245-261.
- Stöver, B. C., and Müller, K. F. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinf.* 11:7.
- Suharjo, R., Sawada, H., and Takikawa, Y. 2014. Phylogenetic study of Japanese *Dickeya* spp. and development of new rapid identification methods using PCR-RFLP. *J. Gen. Plant Pathol.* 80:237-254.
- Toth, I. K., van der Wolf, J. M., Saddler, G., Lojkowska, E., Hélias, V., Pirhonen, M., Tsrör, L., and Elphinstone, J. G. 2011. *Dickeya* species an emerging problem for potato production in Europe. *Plant Pathol.* 60:385-399.
- Tsrör (Lahkim), L., Erlich, O., Hazanovsky, M., Ben Daniel, B., Zig, U., and Lebiush, S. 2012. Detection of *Dickeya* spp. latent infection in potato seed tubers using PCR or ELISA and correlation with disease incidence in commercial field crops under hot-climate conditions. *Plant Pathol.* 61:161-168.
- Tsrör (Lahkim), L., Erlich, O., Lebiush, S., Hazanovsky, M., Zig, U., Sławiak, M., Grabe, G., van der Wolf, J. M., and van de Haar, J. J. 2009. Assessment of recent outbreaks of *Dickeya* sp. (syn. *Erwinia chrysanthemi*) slow wilt in potato crops in Israel. *Eur. J. Plant Pathol.* 123:311-320.
- van der Wolf, J. M., Nijhuis, E. H., Kowalewska, M. J., Saddler, G. S., Parkinson, N., Elphinstone, J. G., Pritchard, L., Toth, I. K., Lojkowska, E., Potrykus, M., Waleron, M., de Vos, P., Cleenwerck, I., Pirhonen, M., Garland, L., Hélias, V., Pothier, J. F., Pflüger, V., Duffy, B., Tsrör, L., and Manulis, S. 2014. *Dickeya solani* sp. nov., a pectinolytic plant pathogenic bacterium isolated from potato (*Solanum tuberosum*). *Int. J. Syst. Evol. Microbiol.* 64:768-774.
- Waleron, M., Waleron, K., Podhajska, A. J., and Lojkowska, E. 2002. Genotyping of bacteria belonging to the former *Erwinia* genus by PCR-RFLP analysis of a *recA* gene fragment. *Microbiology* 148:583-595.
- Zhang, J., Shen, H., Pu, X., Lin, B., and Hu, J. 2014. Identification of *Dickeya zeae* as a causal agent of bacterial soft rot in banana in China. *Plant Dis.* 98:436-442.